

Clinical Utility Validation of an Automated Ultrarapid Gene Fusion Assay for NSCLC



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Received 12 July 2022; revised 13 September 2022; accepted 4 November 2022

Available online - 9 November 2022

ABSTRACT

Introduction: Gene rearrangements are frequent oncologic drivers in NSCLC, and many are suitable for treatment with Food and Drug Administration–approved or experimental targeted therapies. We evaluated the accuracy, specimen acceptance profile, and limits of detection of a rapid fusion assay (Idylla GeneFusion Assay), a commercially available ultrarapid molecular assay, for its clinical utility.

Methods: A collection of 97 specimens which had previously undergone next-generation sequencing testing were analyzed using the rapid fusion assay. Accuracy was evaluated by sensitivity and specificity compared with the next-generation sequencing results. The performance characteristics were tested by using a variety of different clinically relevant specimen types. Limits of detection were assessed by evaluating different input of tumor percentage and material amount.

Results: The rapid fusion assay was found to have 100% sensitivity in detecting fusions of *ALK*, *ROS1*, *RET*, *NTRK1*, and *MET* exon 14 skipping and 83% sensitivity for *NTRK2/3* fusions. There were 100% specificity in detecting fusions of *ROS1*, *RET*, *NTRK2/3*, and *MET* exon 14 skipping and 98% specificity for *ALK*. Testing was successful with formalin-fixed paraffin-embedded biopsy and surgical tissues, cell blocks from fine-needle aspiration and pleural fluid (down to 5% tumor content, 18 mm² tissue scraped), cytology smears (≥ 300 cells), and previously extracted RNA (minimal 20 ng).

Conclusions: The rapid fusion assay is quick, accurate, and versatile, allowing reliable detection of *ALK*, *ROS1*, *RET* fusions, and *MET* exon 14 skipping in NSCLC, and *NTRK* fusions. Rapid molecular testing may expedite treatment with appropriate targeted therapies.

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Keywords: Gene fusions; Rapid testing; Non–small cell lung cancer; Targeted therapy; Idylla

Introduction

NSCLC represents the largest category of lung cancers, with adenocarcinoma being the most common.^{1,2} The discovery of oncologic drivers and development of targeted therapies have substantially improved patient outcomes.^{1,3} Several gene rearrangements and splicing alterations involving tyrosine kinase receptors are suitable for treatment with targeted therapies.^{4–7} Indeed, multiple small molecules have been approved by the Food and Drug Administration targeting *ALK* (approximately 4% of NSCLC), *ROS1* (approximately 2%), *RET* (approximately 2%), and *NTRK* (approximately 0.1%) rearrangements, and *MET* exon 14 skipping (approximately 3%).^{1,8} Overall, these molecular alterations account for approximately 11% of potentially targetable

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Disclosure: The Mayo Clinic received sponsorship of Idylla GeneFusion Assay cartridges from Biocartis.

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Cite this article as: Buglioni A, Caffes PL, Hessler MG, Mansfield AS, Lo YC. Clinical utility validation of an automated ultrarapid gene fusion assay for NSCLC. *JTO Clin Res Rep.* 2022;3:100434.

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ISSN: 2666-3643

<https://doi.org/10.1016/j.jtocrr.2022.100434>

NSCLC. As targeted therapies are primarily approved for the treatment of patients with metastatic disease, the rapid detection of these alterations can potentially expedite treatment decision making for patients who have symptoms and are in need of the most effective therapy.

Accuracy and feasibility of detecting such alterations has been challenging. Routine testing by multiple stand-alone single-gene assays, such as immunohistochemistry and fluorescence in situ hybridization (FISH), is not recommended owing to concern for tissue exhaustion. Next-generation sequencing (NGS) panels, ideally including RNA-based component, are generally preferred because they may assess the presence of all relevant mutations and are more cost effective than multiple single-gene tests.^{9–12} Nevertheless, the long turnaround time (2–4 wk) dampens their clinical value in scenarios where timely decisions for patient management must be made. In these instances, a rapid and accurate assay with minimal tissue requirement is strongly desired.

Beta testing reports of an ultrarapid gene fusion assay, using mainly prototype cartridges, revealed encouraging accuracy results.^{13–15} In the current study, we evaluated the clinical utility of this recently commercially available assay, using the manufactured kits. We tested its performance of accuracy, tissue acceptance profile, and limit of detection for capturing clinically relevant gene fusions involving *ALK*, *ROS1*, *RET*, *NTRK1/2/3* genes, and *MET* exon 14 skipping alterations.

Materials and Methods

This study was deemed exempt by the Mayo Clinic Institutional Review Board, and patient's informed consent was obtained.

Assay

The Idylla GeneFusion Assay (Biocartis, Mechelen, Belgium) is a rapid and automated cartridge-based system optimized for formalin-fixed, paraffin-embedded (FFPE) tumor tissue.^{13,14} RNA extraction, amplification, multiplex quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR), and data analysis are performed within a single cartridge-based workflow. It detects fusions by the following two methods: (1) fusion-specific (FS) detection and (2) expression imbalance (EI) analysis. FS detection includes RT-qPCR primers designed specifically for 17 *ALK*, 13 *ROS1*, and seven *RET* rearrangements, and primers designed for the *MET* exon 13-exon 15 junction. The EI method detects fusion events by analyzing expression difference between the 3' (kinase domain) and 5' ends of the mRNA of *ALK*, *ROS1*, *RET*, and *NTRK1/2/3*.

Samples

A total of 97 tumor samples from 87 unique patients with prior clinical NGS profiling were included. There were 64 cases with NSCLC, including 44 samples with either *ALK*, *ROS1*, or *RET* fusion, or *MET* exon 14 skipping alterations. There were 20 NSCLC samples negative for gene rearrangements. An additional 33 samples had a fusion involving *NTRK1/2/3*, predominantly representing sarcomas and central nervous system tumors. Accuracy was evaluated for each individual gene tested, compared with the NGS results. The reference NGS testing included five NGS panels currently used at the Mayo Clinic, which are as follows: Lung Panel with Tumor Rearrangement, MayoComplete Solid Tumor Panel, Sarcoma Targeted Gene Fusion Panel, NTRK Gene Fusion Panel, and Neuro-Oncology Expanded Panel. Detailed information can be found in [Supplementary Table 1](#).

Specimen-Type Acceptance Studies

For the NSCLC samples, a wide range of material types were included, which are as follows: 33 FFPE surgical specimens, 10 fine-needle aspiration (FNA) or pleural fluid in cell blocks (also FFPE), 10 FNA smears stained with Diff-Quik or Pap, and 11 cases of previously extracted RNA. Samples positive for *NTRK1/2/3* fusions were all FFPE specimens.

Limit of Detection Studies, Variable Parameters

To assess minimum tumor percentage input requirement, a pathologist used a hematoxylin and eosin slide to mark areas with approximately 10% or 5% tumor cell content for three different samples. Unstained FFPE sections were scraped accordingly and tested.

For minimal size of tumor tissue required, four FFPE samples were each scraped at areas of 72 mm², 36 mm², and 18 mm² and tested. To evaluate the cellularity requirement for smears, two samples with high cellularity (>5000 cells on a slide), four samples with medium cellularity (3000–5000 cells), and four samples with low cellularity (300–3000 cells) were run, all containing at least 5% tumor cell content. To determine the lowest acceptance limit of nucleic acid, three different samples were each tested at RNA amounts of 20, 10, and 5 ng.

Results

Accuracy

All 12 samples (100%) with known *ALK* fusion were correctly detected by this assay, including 11 by FS detection and one by EI analysis ([Table 1](#)). Ten samples with *ROS1* fusion (100%) were correctly detected, all by the FS method. All 12 *RET*-rearranged cases (100%)

Table 1. Accuracy Summary of Fusion Detection of Each Gene

Gene	Number of Positive Samples	Number of Samples Detected by FS Method	Number of Samples Detected by EI Only	Sensitivity	Specificity ^a
<i>ALK</i>	12	11	1	12/12 (100%)	51/52 (98%)
<i>ROS1</i>	10	10	0	10/10 (100%)	54/54 (100%)
<i>RET</i>	12	11	1	12/12 (100%)	52/52 (100%)
<i>MET</i> exon 14 skipping	10	10	N/A	10/10 (100%)	54/54 (100%)
20 samples negative for fusion or <i>MET</i> exon 14 skipping					
<i>NTRK1</i>	10	N/A	10	10/10 (100%)	87/87 (100%) ^b
<i>NTRK2</i>	10	N/A	1	1/10 (10%)	36/36 (100%) ^b
<i>NTRK3</i>	13	N/A	13	13/13 (100%)	32/37 (86%) ^b
<i>NTRK2/3</i>	23	N/A	19	19/23 (83%)	26/26 (100%) ^b

Note: Samples positive for *ALK*, *ROS1*, *RET* fusions and *MET* exon 14 skipping and negative controls are NSCLC specimens

Samples positive for *NTRK1/2/3* are non-NSCLC specimens

^aDifferent NGS panels (served as reference method) have different gene coverage.

^bIncluding NSCLC samples.

EFS, fusion specific; I, expression imbalance; N/A, not applicable.

were successfully detected (11 FS, one EI). Ten samples with *MET* exon 14 skipping were all (100%) detected. The sensitivities of detecting *ALK*, *ROS1*, and *RET* fusions and *MET* exon 14 skipping in the tested NSCLC samples are all 100%. All results were available in approximately 3 hours.

Of the 10 *NTRK1* fusion-positive samples, all (100%) were successfully detected by this assay. Among the 10 samples positive for *NTRK2* fusion, only one (10%) produced a *NTRK2* fusion-detected result. Surprisingly, five samples were classified as detection of *NTRK3* fusion, whereas four samples had no fusion detected. All 13 (100%) *NTRK3* fusion-positive samples were accurately detected.

This assay did not detect fusions in the 20 fusion-negative NSCLC samples. Because each sample run contained multiplex RT-qPCR reactions evaluating multiple gene rearrangements, a sole detection of one gene fusion implied negative results in other genes. One false-positive detection of *ALK* fusion was called by EI analysis only in a *RET*-rearranged sample. For this sample, break apart *ALK* FISH found no *ALK* gene rearrangement but 80% of the nuclei had three to five copies of the *ALK* gene. In the tested NSCLC samples, the overall specificities for *ALK*, *ROS1*, and *RET* fusions and *MET* exon 14 skipping alteration detection were 98% (51 of 52), 100% (54 of 54), 100% (52 of 52), and 100% (54 of 54), respectively.

No false-positive calls of *NTRK1* or *NTRK2* fusion were noted in the tested samples. The individual specificities of *NTRK1*, *NTRK2*, and *NTRK3* fusion detection were 100% (87 of 87), 100% (36 of 36), and 86% (32 of 37), respectively. If reported in combination as *NTRK2/3* fusion, the detection specificity was 100% (26 of 26) with the sensitivity of 83% (19 of 23).

Specimen-Type Acceptance

Various specimen types were validated. For the 64 NSCLC samples, the concordances were 100% across every material type evaluated (Table 2), except for a false-positive *ALK* fusion call on a *RET*-rearranged case from an FFPE tissue block. Most samples were less than 4 years old. Rearrangement events were concordantly detected in two samples older than 5 years and in four samples collected between 4 and 5 years ago.

Limits of Detection

All three cases produced concordant results when areas of either 10% or 5% tumor content were scraped and tested. Tumor size limit of detection studies revealed that all four samples were concordant when 72, 36, or 18 mm² of the tissue was scraped. All cytology smears yielded concordant results, including one *ROS1*-rearranged case detected at 5% tumor content and low cellularity (300–3000 cells). Finally, three of three RNA samples were concordant at 20 ng, one of three was concordant at 10 ng, but all three samples were invalid when 5 ng was used. These findings indicated that 20 ng of RNA input is required when using pre-extracted RNA (Table 3).

Discussion

In clinical settings where a timely therapeutic decision must be made, rapid molecular testing is desired. The ultraquick turnaround time of the rapid fusion assay, yielding results in approximately 3 hours, can have impactful clinical utility. For patients with NSCLC who have rapid disease progression, quick molecular testing for gene fusions, *EGFR* mutation status, and programmed death-ligand 1 immunohistochemistry can provide valuable information for immediate treatment

Table 2. Summary of Testing Results of Samples With Different Specimen Types

Specimen Type	Number of Samples	Positive Cases by NGS	Positive Cases by Idylla	Negative Cases by NGS	Negative Cases by Idylla	Concordance of Idylla vs. NGS
FFPE tissue block	33	23	23 ^a	10	10	33/33 (100%)
FNA or pleural fluid in cell block (FFPE)	10	7	7	3	3	10/10 (100%)
Cytology/FNA smear	10	7	7	3	3	10/10 (100%)
Pre-extracted RNA	11	7	7	4	4	11/11 (100%)

^aA *RET*-rearranged sample revealed both *RET* and *ALK* fusions detected by Idylla.

FFPE, formalin-fixed, paraffin-embedded; FNA, fine-needle aspiration; NGS, next-generation sequencing.

options. Furthermore, surgical candidates considered for neoadjuvant immunotherapy¹⁶ would benefit from timely exclusion of oncogenic mutations and fusions.

We found that the rapid fusion assay has robust sensitivities and specificities in detecting *ALK*, *ROS1*, and *RET* fusions, and *MET* exon 14 skipping in the NSCLC samples. The detection of *ALK*, *ROS1*, and *RET* fusions includes dual FS and EI methods in this assay. The FS detection covers common fusion partners (e.g., *EML4::ALK* and *CD74::ROS1*). Using the FS method only, regardless of the findings by EI, 94% (32 of 34) of the positive cases were successfully detected by this method with 100% (52 of 52) specificity. The EI analysis provided additional detection covering fusions with uncommon partners or exon combinations. Determining fusion detection by either FS or EI, as suggested by the manufacturer, increases the sensitivity to 100% (34 of 34) but slightly decreases the specificity to 98% (51 of 52). According to the manufacturer, the EI algorithm was designed with a higher threshold; indeed, among 32 samples with fusion detection by the FS method, only 22 were called positive by the EI method. Nevertheless, using the EI method to complement the FS method successfully increases the sensitivity, as it was designed to identify novel fusions. However, one false-positive case was revealed in our study. Thus, in clinical practice, we recommend confirmatory testing (i.e., FISH or NGS) when fusions are detected by the EI method only.

This assay detects *NTRK1/2/3* fusions by the EI method only without the FS method. It revealed an acceptable accuracy in detecting *NTRK* fusions. Using non-NSCLC samples, it revealed excellent detection rate for *NTRK1* and *NTRK3* fusions but not for detection of *NTRK2* fusions. Among the 10 samples known to have *NTRK2* fusion, only one produced a positive result by this assay, whereas five were misidentified as *NTRK3* fusion detected. Importantly, the assay was developed with a limited number of *NTRK2*-rearranged specimens.^{13–15} Furthermore, it has been recently reported that baseline tyrosine kinase gene expression differs among cancer types: (1) *NTRK3* displayed higher expression in glioblastoma and colorectal cancer

compared with other kinase receptors, such as *ALK*, *ROS1*, *RET*, and *NTRK1*; and (2) *NTRK2* had strong difference in baseline expression between adenocarcinoma and squamous cell carcinoma of the lung.¹⁵ These factors could have affected the *NTRK2* and *NTRK3* EI method-calling algorithm. Because *NTRK* fusions are rare in NSCLC (approximately 0.1%–0.3%)⁷ and treatments among *NTRK1/2/3* fusions are the same, we suggest reporting *NTRK1* and combined *NTRK2/3* findings, with confirmatory NGS or FISH testing.

This automated system revealed wide specimen acceptance profile and a simple workflow. It is common for thoracic oncology molecular testing to encounter low tumor percentage or limited-size tissues from bronchoscopy biopsy and FNA specimens. Although NGS RNA panel assay generally requires at least 20% of tumor content and 10 to 15 unstained slides, this assay can reliably detect gene fusions on samples with as little as 5% tumor content with only one to three unstained slides (Table 3). We successfully validated a wide range of tissue types, including tissue FFPE, FNA, and pleural fluid cell block FFPE, cytology smears, and extracted RNA, expanding its practical clinical utility. In the current practice, a significant number of non-small cell carcinoma diagnoses are made by bronchoscopy procedures. However, cytology specimens have not been thoroughly validated in molecular tests nor in immunohistochemistry studies. Here, we provided validation data to support the reliable clinical utility of this assay to accept various cytology specimens, including FNA, smears, and fluid cell block preparation. Finally, the automated workflow of this assay requires minimal hands-on time, saving labor cost for molecular laboratories.

In conclusion, our study reveals the rapid fusion assay as a fast and reliable alternative assay for detecting targetable *ALK*, *ROS1*, and *RET* fusions, and *MET* exon 14 skipping in the setting of NSCLC and detection of the less common *NTRK1/2/3* fusions. In the clinical setting, this assay offers timely and impactful molecular information for managing patients with NSCLC with targeted therapy and selecting patients for neoadjuvant therapy or clinical

Table 3. Limit of Detection of Idylla GeneFusion Assay and Comparison With Large Panel NGS Assays

Characteristic	Suggested Requirement for NGS ^a	Minimum Requirement for NGS ^a	Suggestion Requirement by Manufacturer for Idylla	Minimum Requirement for Idylla After Verification
Tissue size	72 mm ²	36 mm ²	20 mm ²	18 mm ²
Tumor %	40%	20%	10%	5%
Total cells on smear	At least 5000 cells	3000 cells	N/A	3000 cells, possible >300 cells
RNA, total	80 ng	80 ng	N/A	20 ng

^aUsing clinically validated MayoComplete Solid Tumor Panel for reference. N/A, not available; NGS, next-generation sequencing.

trials. Rapid molecular testing could be considered before larger comprehensive gene panel testing to expedite patient care.

CRediT Authorship Contribution Statement

Alessia Buglioni: Data curation, Writing—original draft, Writing—review and editing.

Patricia L. Caffes: Data curation, Methodology, Validation, Writing—review and editing.

Mark G. Hessler: Data curation, Methodology, Validation, Writing—review and editing.

Aaron S. Mansfield; Writing—review and editing.

Ying-Chun Lo: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing—review and editing.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at <https://doi.org/10.1016/j.jtocrr.2022.100434>.

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